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Gantt Lake
Covington County, Alabama

Pacific Northwest Environmental Research Lab, Corvallis, Oreg

Prepared for

Alabama Water Improvement Commission, Montgomery

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16. ABSTRACT
Annual total phosphorus and total nitrogen loadings to the lake were estimated and subdivided according to either point or non-point source origin. An assessment of the lake's trophic condition and limiting nutrient is also provided. All data collected by the U.S.E.P.A. National Eutrophication Survey during the one year study of the lake and its tributaries are included in the report.

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NATIONAL EUTROPHICATION SURVEY

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REPORT
ON
GANTT LAKE
COVINGTON COUNTY
ALABAMA

EPA REGION IV

WORKING PAPER NO. 227

WITH THE COOPERATION OF THE

ALABAMA WATER IMPROVEMENT COMMISSION

AND THE

ALABAMA NATIONAL GUARD

JULY 1976

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FOREWORD

The National Eutrophication Survey was initiated in 1972 in response to an Administration commitment to investigate the nationwide threat of accelerated eutrophication to freshwater lakes and reservoirs.

OBJECTIVES

The Survey was designed to develop, in conjunction with state environmental agencies, information on nutrient sources, concentrations, and impact on selected freshwater lakes as a basis for formulating comprehensive and coordinated national, regional, and state management practices relating to point source discharge reduction and nonpoint source pollution abatement in lake watersheds.

ANALYTIC APPROACH

The mathematical and statistical procedures selected for the Survey's eutrophication analysis are based on related concepts that:

a. A generalized representation or model relating sources, concentrations, and impacts can be constructed.

b. By applying measurements of relevant parameters associated with lake degradation, the generalized model can be transformed into an operational representation of a lake, its drainage basin, and related nutrients.

c. With such a transformation, an assessment of the potential for eutrophication control can be made.

LAKE ANALYSIS

In this report, the first stage of evaluation of lake and watershed data collected from the study lake and its drainage basin is documented. The report is formatted to provide state environmental agencies with specific information for basin planning [§303(e)], water quality criteria/standards review [§303(c)], clean lakes [§314(a,b)], and water quality monitoring [§106 and §305(b)] activities mandated by the Federal Water Pollution Control Act Amendments of 1972.

Beyond the single lake analysis, broader based correlations between nutrient concentrations (and loading) and trophic condition are being made to advance the rationale and data base for refinement of nutrient water quality criteria for the Nation's freshwater lakes. Likewise, multivariate evaluations for the relationships between land use, nutrient export, and trophic condition, by lake class or use, are being developed to assist in the formulation of planning guidelines and policies by the U.S. Environmental Protection Agency and to augment plans implementation by the states.

ACKNOWLEDGEMENTS

The staff of the National Eutrophication Survey (Office of Research and Development, U.S. Environmental Protection Agency) expresses sincere appreciation to the Alabama Water Improvement Commission for professional involvement and to the Alabama National Guard for conducting the tributary sampling phase of the Survey.

E. John Williford, Chief, Surveillance and Monitoring Section; and John C. Chitwood, Biologist, Surveillance and Monitoring Section; and Sam L. Coleman, Water Quality Planning Section; and M. H. Floyd, Engineer, Surveillance and Monitoring Section; and Truman Green, Engineer, Municipal Waste Control Section; and Tim McCartha, Biologist, Surveillance and Monitoring Section; and James E. McIndoe, Engineer, Water Quality Planning Section; and Richard T. Maddox, Engineer, Industrial Waste Control Section; and James T. White, Engineer, Municipal Waste Control Section provided invaluable lake documentation and counsel during the course of the Survey.

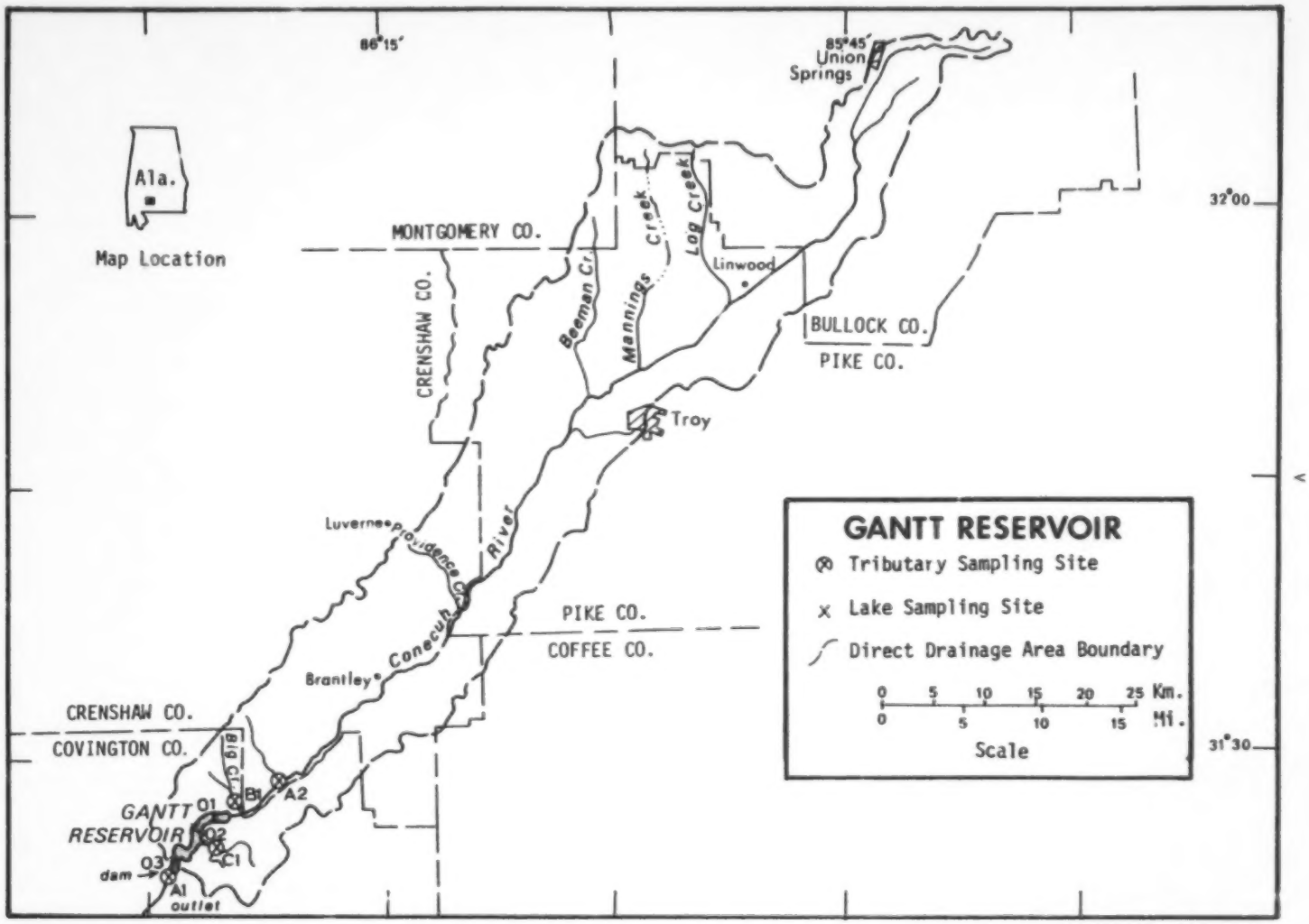
Major General Charles A. Rollo, Adjutant General of Alabama, and Project Officer Lt. Col. Wash B. Ray, who directed the volunteer efforts of the Alabama National Guardsmen, are also gratefully acknowledged for their assistance to the Survey.

NATIONAL EUTROPHICATION SURVEY

STUDY LAKES

STATE OF ALABAMA

LAKE NAME	COUNTY
Bankhead	Walker
Gantt	Covington
Guntersville	Marshall, Johnson
Holt Lock and Dam	Tuscaloosa
Lay	Chilton, Coosa
Martin	Elmore, Tallapoosa
Mitchell	Coosa, Chilton
Pickwick	Colbert, Lauderdale (Tishomingo in MS and Hardin in TN)
Purdy	Jefferson, Shelby
Weiss	Cherokee
Wilson	Lauderdale, Colbert, Lawrence


86°15'
85°45'
Union Springs
Ala.
Map Location
MONTGOMERY CO.
CRENSHAW CO.
Beeman Cr.
Mannings Creek
Log Creek
Linwood
32°00
BULLOCK CO.
PIKE CO.
Troy
Luverne
Providence Cr.
Conecuh River
PIKE CO.
COFFEE CO.
Brantley
CRENSHAW CO.
COVINGTON CO.
Big Cr.
GANTT RESERVOIR
dam
outlet
Q1
Q2
Q3
B1
A2
C1
A1
31°30
GANTT RESERVOIR
Tributary Sampling Site
Lake Sampling Site
Direct Drainage Area Boundary
0 5 10 15 20 25 Km.
0 5 10 15 Mi.
Scale

GANTT RESERVOIR, ALABAMA

STORET NO. 0103

I. CONCLUSIONS

A. Trophic Condition:*

Survey data indicate Gantt Reservoir is mesotrophic-eutrophic. The lake is characterized by low Secchi disc transparency and chlorophyll a values, and low potential for primary productivity as measured by algal assay control yields. Oxygen depression occurred below 4.6 m during August sampling of the lake.

Nutrient concentrations in the reservoir were moderately low, with total phosphorus not exceeding 0.054 mg/liter and inorganic nitrogen not exceeding 0.410 mg/liter in any of the samples. The increase in total phosphorus values during June sampling is probably a result of spring flooding, which would increase the levels of inorganic phosphorus in the water.

B. Rate-Limiting Nutrient:

Algal assay results indicate Gantt Reservoir was initially limited by phosphorus at the time of sample collection, but became nitrogen limited at higher phosphorus concentrations. Additions of nitrogen alone did not

*See Appendix E.

stimulate a growth response. The ratios of available inorganic nitrogen to orthophosphorus (N/P) in sampled waters (51/1, 33/1, and 22/1 for spring, summer, and fall sampling, respectively) were generally consistent with phosphorus limitation.

C. Nutrient Controllability:

1. Point sources -

There were no known point sources impacting Gantt Reservoir during the sampling year. The present loading rate of 2.68 g $P/m^2/yr$ is about twice that proposed by Vollenweider (1975) as a eutrophic rate. However, loading calculations based upon available nutrient concentrations and flow data yield a net export of both nitrogen and phosphorus from the reservoir, suggesting sampling was not adequate to depict actual loading and export rates. The A(2) inlet sampling site was approximately 8.0 km north of the actual lake inlet. Nutrient loading from the watershed surrounding that reach of Conecuh River has been omitted from lake loading calculations. The export could also be attributed to undetected discharges reaching the lake from unknown industrial or municipal sources.

Point sources from the cities of Union Springs and Troy are known to impact the Conecuh River upstream from Gantt Reservoir. These sources are not included in this report in the Gantt Reservoir nutrient budget due to their distance from the lake. However, it is expected that the high background loading (page 14) for the Conecuh River is due in part to unmeasured contributions from these sources.

2. Nonpoint sources -

The mean annual phosphorus loads from the three gaged tributaries amounted to 97.2% of the total load reaching the lake. The estimated phosphorus load from the ungaged drainage areas amounted to 2.1% of the total load. Additional sampling and an evaluation of current land use and lakeshore construction are required before a nutrient budget for the lake can be determined.

II. LAKE AND DRAINAGE BASIN CHARACTERISTICS

Lake and drainage basin characteristics are itemized below. Lake surface area and mean depth were provided by the Alabama Water Improvement Commission; maximum depth was estimated on the basis of National Eutrophication Survey (NES) sampling data; tributary flow data were provided by the Alabama District Office of the U.S. Geological Survey (USGS)(outlet drainage area includes the lake surface area). Mean hydraulic retention time was obtained by dividing the lake volume by mean flow of the outlet. Precipitation values were estimated by methods as outlined in NES Working Paper No. 175. A table of metric/English conversions is included as Appendix A.

A. Lake Morphometry:

1. Surface area: 11.20 km^2.
2. Mean depth: 1.4 meters.
3. Maximum depth: 10.7 meters.
4. Volume: 15.680 x 10^6 m^3.
5. Mean hydraulic retention time: 7 days.

B. Tributary and Outlet (see Appendix B for flow data):

1. Tributaries -

Name	Drainage area(km^2)	Mean flow (m^3/sec)
A(2) Conecuh River	1,489.2	21.52
B(1) Big Creek	25.9	0.19
C(1) Feagin Creek	45.3	0.49
Minor tributaries and immediate drainage -	104.1	2.01
Totals	1,664.5	24.21
2. Outlet - A(1) Conecuh River	1,675.7	27.56

C. Precipitation:

1. Year of sampling: 179.2 cm.

2. Mean annual: 146.4 cm.

III. LAKE WATER QUALITY SUMMARY

Gantt Reservoir was sampled three times during the open-water season of 1973 by means of a pontoon-equipped Huey helicopter. Each time, samples for physical and chemical parameters were collected from three stations on the lake and from a number of depths at each station (see map, page v). During each visit, depth-integrated samples were collected from each station for chlorophyll a analysis and phytoplankton identification and enumeration. During the first visit, a single 18.9-liter depth-integrated sample was composited for algal assays. Maximum depths sampled were 3.4 m at Station 1, 4.6 m at Station 2, and 8.8 m at Station 3. For a more detailed explanation of NES methods, see NES Working Paper No. 175.

The results obtained are presented in full in Appendix C and are summarized in III-A for waters at the surface and at the maximum depth for each site. Results of the phytoplankton counts and chlorophyll a determinations are included in III-B. Results of the limiting nutrient study are presented in III-C.

GANTT RESERVOIR
STORET CODE 0103

PHYSICAL AND CHEMICAL CHARACTERISTICS

PARAMETER		(6/19/73) S*** = 3				(8/30/73) S*** = 3				(11/ 3/73) S*** = 3			
	N*	RANGE	MEDIAN	MAX DEPTH RANGE (METERS)		N*	RANGE	MEDIAN	MAX DEPTH RANGE (METERS)	N*	RANGE	MEDIAN	MAX DEPTH RANGE (METERS)
TEMPERATURE (DEG CENT)													
0.-1.5 M DEPTH	4	25.6- 28.8	27.7	0.0- 1.2		5	29.2- 31.1	30.2	0.0- 1.5	6	18.8- 21.4	18.9	0.0- 1.5
MAX DEPTH**	3	25.2- 25.8	25.6	1.2- 8.8		3	27.5- 28.3	28.2	1.8- 7.6	3	15.9- 19.0	17.9	3.0- 7.6
DISSOLVED OXYGEN (MG/L)													
0.-1.5 M DEPTH	1	5.4- 5.4	5.4	1.2- 1.2		3	5.8- 6.0	5.4	0.0- 1.5	3	8.0- 8.6	8.2	1.5- 1.5
MAX DEPTH**	3	3.2- 5.4	5.0	1.2- 8.8		3	1.8- 6.6	2.6	1.8- 7.6	3	8.4- 9.0	8.6	3.0- 7.6
CONDUCTIVITY (UMHOS)													
0.-1.5 M DEPTH	4	45.- 47.	46.	0.0- 1.2		5	60.- 70.	63.	0.0- 1.5	6	64.- 87.	68.	0.0- 1.5
MAX DEPTH**	3	40.- 48.	47.	1.2- 8.8		3	68.- 73.	70.	1.8- 7.6	3	64.- 87.	69.	3.0- 7.6
PH (STANDARD UNITS)													
0.-1.5 M DEPTH	4	6.8- 6.9	6.8	0.0- 1.2		5	7.3- 8.0	7.6	0.0- 1.5	6	7.3- 7.8	7.5	0.0- 1.5
MAX DEPTH**	3	6.7- 6.8	6.8	1.2- 8.8		3	7.1- 7.8	7.4	1.8- 7.6	3	7.3- 7.7	7.5	3.0- 7.6
TOTAL ALKALINITY (MG/L)													
0.-1.5 M DEPTH	4	19.- 33.	20.	0.0- 1.2		5	17.- 24.	20.	0.0- 1.5	6	22.- 34.	26.	0.0- 1.5
MAX DEPTH**	3	17.- 20.	20.	1.2- 8.8		3	18.- 25.	23.	1.8- 7.6	3	23.- 37.	28.	3.0- 7.6
TOTAL P (MG/L)													
0.-1.5 M DEPTH	4	0.045-0.052	0.047	0.0- 1.2		5	0.022-0.027	0.023	0.0- 1.5	6	0.016-0.032	0.020	0.0- 1.5
MAX DEPTH**	3	0.049-0.054	0.049	1.2- 8.8		3	0.025-0.029	0.027	1.8- 7.6	3	0.019-0.037	0.029	3.0- 7.6
DISSOLVED ORTHO P (MG/L)													
0.-1.5 M DEPTH	4	0.005-0.012	0.008	0.0- 1.2		5	0.007-0.010	0.009	0.0- 1.5	6	0.007-0.014	0.008	0.0- 1.5
MAX DEPTH**	3	0.005-0.006	0.006	1.2- 8.8		3	0.006-0.013	0.008	1.8- 7.6	3	0.008-0.010	0.009	3.0- 7.6
NO2+NO3 (MG/L)													
0.-1.5 M DEPTH	4	0.180-0.200	0.195	0.0- 1.2		5	0.110-0.180	0.130	0.0- 1.5	6	0.120-0.250	0.150	0.0- 1.5
MAX DEPTH**	3	0.200-0.200	0.200	1.2- 8.8		3	0.090-0.160	0.130	1.8- 7.6	3	0.130-0.280	0.140	3.0- 7.6
AMMONIA (MG/L)													
0.-1.5 M DEPTH	4	0.140-0.190	0.160	0.0- 1.2		5	0.120-0.180	0.130	0.0- 1.5	6	0.040-0.070	0.050	0.0- 1.5
MAX DEPTH**	3	0.140-0.210	0.170	1.2- 8.8		3	0.100-0.300	0.200	1.8- 7.6	3	0.040-0.060	0.050	3.0- 7.6
KJELDAHL P (MG/L)													
0.-1.5 M DEPTH	4	0.400-0.600	0.550	0.0- 1.2		5	0.400-0.700	0.500	0.0- 1.5	6	0.200-1.000	0.300	0.0- 1.5
MAX DEPTH**	3	0.400-0.400	0.400	1.2- 8.8		3	0.300-0.600	0.400	1.8- 7.6	3	0.200-0.200	0.200	3.0- 7.6
SECCHI DISC (METERS)													
	3	0.5- 0.6	0.5			3	0.8- 1.0	0.9		3	0.9- 1.5	1.2	

* N = NO. OF SAMPLES
** MAXIMUM DEPTH SAMPLED AT EACH SITE
*** S = NO. OF SITES SAMPLED ON THIS DATE

B. Biological Characteristics:

1. Phytoplankton -

Sampling Date	Dominant Genera	Algal Units per ml
06/19/73	1. Flagellates	170
	2. Dinoflagellates	58
	3. Melosira	25
	4. Pennate diatom	25
	5. Oscillatoria	17
	Other genera	104
	Total	399
08/30/73	1. Flagellates	56
	2. Lyngbya	41
	3. Oscillatoria	32
	4. Dactylococcopsis	24
	5. Nitzschia	16
	Other genera	67
	Total	236
11/03/73	1. Flagellates	350
	2. Dactylococcopsis	110
	3. Centric diatom	46
	4. Nitzschia	46
	5. Oscillatoria	37
	Other genera	92
	Total	681

2. Chlorophyll *a* -

Sampling Date	Station Number	Chlorophyll *a* (µg/liter)
06/19/73	1	2.7
	2	2.4
	3	2.1
08/30/73	1	3.0
	2	1.7
	3	4.0
11/03/73	1	0.9
	2	1.1
	3	1.4

C. Limiting Nutrient Study:

1. Autoclaved, filtered, and nutrient spiked -

Spike(mg/l)	Ortho P Conc.(mg/l)	Inorganic N Conc.(mg/l)	Maximum Yield (mg/l-dry wt.)
Control	0.008	0.104	0.1
0.010 P	0.018	0.104	1.4
0.020 P	0.028	0.104	2.6
0.050 P	0.058	0.104	2.5
0.025 P + 0.5 N	0.033	0.604	10.2
0.050 P + 1.0 N	0.058	1.104	20.8
1.0 N	0.008	1.104	0.1

2. Filtered and nutrient spiked -

Spike(mg/l)	Ortho P Conc.(mg/l)	Inorganic N Conc.(mg/l)	Maximum Yield (mg/l-dry wt.)
Control	0.005	0.124	0.1
0.010 P	0.015	0.124	2.0
0.020 P	0.025	0.124	3.1
0.050 P	0.055	0.124	3.3
0.025 P + 0.5 N	0.030	0.624	11.3
0.050 P + 1.0 N	0.055	1.124	21.6
1.0 N	0.005	1.124	0.1

3. Discussion -

The control yield of the assay alga, Selenastrum capricornutum, indicates that the potential for primary production in Gantt Reservoir was low at the time of sample analysis. An increase in yield with increased levels of orthophosphate (to about 0.028 mg/l) as well as a lack of response when only nitrogen was added indicates phosphorus limitation. Maximum growth response was achieved with the simultaneous addition of both phosphorus and nitrogen.

The N/P ratios in the field were greater than 22/1 on all three sampling occasions, further indicating primary limitation by phosphorus in Gantt Reservoir (an N/P ratio of 14/1 or greater generally reflects phosphorus limitation).

IV. NUTRIENT LOADINGS
(See Appendix D for data)

For the determination of nutrient loadings, the Alabama National Guard collected monthly near-surface grab samples from each of the tributary sites indicated (see map), except for the high runoff months of January and February when two samples were collected. Sampling was begun in March 1973, and was completed in February 1974.

Through an interagency agreement, stream flow estimates for the year of sampling and a "normalized" or average year were provided by the Alabama District Office of USGS for the tributary sites nearest the lake.

In this report, nutrient loads for sampled tributaries were determined by using a modification of a USGS computer program for calculating stream loadings. Nutrient loads indicated for tributaries are those measured minus known point source loads, if any.

Nutrient loadings for unsampled "minor tributaries and immediate drainage" ("ZZ" of USGS) were estimated by using the mean annual nutrient loads, in $kg/km^2/yr$, for Big Creek and Feagin Creek at Stations B(1) and C(1), respectively, and multiplying the means by the ZZ drainage area in km^2.

A. Annual Total Phosphorus Loading - Average Year:

1. Inputs -

Source	kg P/yr	% of total
a. Tributaries (nonpoint load) -		
A(2) Conecuh River	28,625	95.5
B(1) Big Creek	105	0.3
C(1) Feagin Creek	405	1.4
b. Minor tributaries and immediate drainage (nonpoint load) -	625	2.1
c. Known municipal STP's - None		
d. Septic tanks* -	20	0.1
e. Known industrial - None		
f. Direct precipitation** -	195	0.6
Totals	29,975	100.0
2. Outputs - A(1) Conecuh River	32,250	
3. Net annual P export***	2,275	

*Estimate based on 70 lakeshore residences.
**Estimated (see NES Working Paper No. 175).
***Export probably due to unknown sources and/or sampling error.

B. Annual Total Nitrogen Loading - Average Year:

1. Inputs -

Source	kg N/yr	% of total
a. Tributaries (nonpoint load) -		
A(2) Conecuh River	673,175	93.1
B(1) Big Creek	4,615	0.6
C(1) Feagin Creek	10,675	1.5
b. Minor tributaries and immediate drainage (nonpoint load) -	21,550	3.0
c. Known municipal STP's - None		
d. Septic tanks* -	745	0.1
e. Known industrial - None		
f. Direct precipitation** -	12,090	1.7
Totals	722,850	100.0

2. Outputs - A(1) Conecuh River 765,985

3. Net annual N export*** - 43,135

*Estimate based on 70 lakeshore residences.
**Estimated (see NES Working Paper No. 175).
***Export probably due to unknown sources and/or sampling error.

C. Mean Annual Nonpoint Nutrient Export by Subdrainage Area:

Tributary	kg P/km^2/yr	kg N/km^2/yr
Conecuh River	19	452
Big Creek	4	178
Feagin Creek	9	236

D. Yearly Loadings:

In the following table, the existing phosphorus annual loading is compared to the relationship proposed by Vollenweider (1975). Essentially, his eutrophic loading is that at which the receiving waters would become eutrophic or remain eutrophic; his oligotrophic loading is that which would result in the receiving water remaining oligotrophic or becoming oligotrophic if morphometry permitted. A mesotrophic loading would be considered one between eutrophic and oligotrophic.

Note that Vollenweider's model may not apply to lakes with short hydraulic retention times or in which light penetration is severely restricted by high concentrations of suspended solids in the surface waters.

	Total Yearly Phosphorus Loading (g/m^2/yr)
Estimated loading for Gantt Reservoir	2.68
Vollenweider's eutrophic loading	1.64
Vollenweider's oligotrophic loading	0.82

V. LITERATURE REVIEWED

U.S. Environmental Protection Agency. 1975. National Eutrophication Survey Methods 1973-1976. National Eutrophication Survey Working Paper No. 175. National Environmental Research Center, Las Vegas, Nevada, and Pacific Northwest Environmental Research Laboratory, Corvallis, Oregon.

Vollenweider, R. A., 1975. Input-Output Models With Final Reference to the Phosphorus Loading Concept in Limnology. Schweiz. Z. Hydrol. 37: 53-84.

VI. APPENDICES

APPENDIX A

CONVERSION FACTORS

CONVERSION FACTORS

Hectares x 2.471 = acres

Kilometers x 0.6214 = miles

Meters x 3.281 = feet

Cubic meters x 8.107×10^{-4} = acre/feet

Square kilometers x 0.3861 = square miles

Cubic meters/sec x 35.315 = cubic feet/sec

Centimeters x 0.3937 = inches

Kilograms x 2.205 = pounds

Kilograms/square kilometer x 5.711 = lbs/square mile

APPENDIX B

TRIBUTARY FLOW DATA

TRIBUTARY FLOW INFORMATION FOR ALABAMA 07/22/76

LAKE CODE 0103 GANTT RESERVOIR

TOTAL DRAINAGE AREA OF LAKE(SQ KM) 1675.7

TRIBUTARY	SUB-DRAINAGE AREA(SQ KM)	NORMALIZED FLOWS(CMS) JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC	MEAN
0103A1	1675.7	38.79	39.36	60.88	49.84	23.50	14.16	18.12	22.37	12.88	9.49	18.69	23.22	27.55
0103A2	1489.2	38.82	36.81	48.14	41.86	20.67	9.63	12.83	10.98	9.49	6.88	11.89	21.88	21.52
0103B1	25.9	0.221	0.269	0.396	0.311	0.161	0.130	0.139	0.116	0.105	0.082	0.142	0.193	0.188
0103C1	45.3	0.57	0.71	0.96	0.79	0.42	0.34	0.40	0.31	0.28	0.23	0.40	0.51	0.49
0103ZZ	115.3	2.32	2.75	3.62	3.00	1.81	1.53	1.64	1.39	1.30	1.05	1.67	2.10	2.91

SUMMARY

TOTAL DRAINAGE AREA OF LAKE = 1675.7
SUM OF SUB-DRAINAGE AREAS = 1675.7

TOTAL FLOW IN = 291.61
TOTAL FLOW OUT = 331.31

MEAN MONTHLY FLOWS AND DAILY FLOWS(CMS)

TRIBUTARY	MONTH	YEAR	MEAN FLOW	DAY	FLOW	DAY	FLOW	DAY	FLOW
0103A1	3	73	64.846	4	2.888				
	4	73	141.584	2	252.869				
	5	73	35.396	2	71.925				
	6	73	32.564	5	2.945				
	7	73	8.778	9	2.698				
	8	73	10.982	6	2.945				
	9	73	5.522	4	3.653				
	10	73	4.446	12	1.388				
	11	73	6.888	6	3.398				
	12	73	16.282	3	8.920				
	1	74	41.059	7	37.378	21	60.881		
	2	74	58.191	6	46.723	14	67.960		
0103A2	3	73	56.917	4	22.512				
	4	73	126.010	2	342.634				
	5	73	31.149	2	82.119				
	6	73	28.317	5	17.556				
	7	73	7.584	9	5.805				
	8	73	9.571	6	8.778				
	9	73	4.672	4	5.097				
	10	73	3.851	12	3.295				
	11	73	5.239	6	3.087				
	12	73	14.017	3	7.646				
	1	74	36.246	7	32.564	21	53.882		
	2	74	50.970	6	41.059	14	59.465		

TRIBUTARY FLOW INFORMATION FOR ALABAMA 07/22/76

LAKE CODE 0103 GANTT RESERVOIR

MEAN MONTHLY FLOWS AND DAILY FLOWS(CMS)

TRIBUTARY	MONTH	YEAR	MEAN FLOW	DAY	FLOW	DAY	FLOW	DAY	FLOW
010381	3	73	0.595	4	0.340				
	4	73	7.362	2	0.736				
	5	73	0.340	2	0.283				
	6	73	0.368	5	0.340				
	7	73	0.198	9	0.252				
	8	73	0.136	6	0.144				
	9	73	0.119	4	0.125				
	10	73	0.079	12	0.068				
	11	73	0.130	6	0.105				
	12	73	0.159	3	0.096				
	1	74	0.396	7	0.178	21	1.897		
	2	74	0.340	6	0.212	14	0.246		
0103C1	3	73	1.472	4	0.623				
	4	73	1.812	2	1.812				
	5	73	0.878	2	0.736				
	6	73	0.963	5	0.906				
	7	73	0.396	9	0.680				
	8	73	0.510	6	0.396				
	9	73	0.272	4	0.311				
	10	73	0.221	12	0.195				
	11	73	0.340	6	0.283				
	12	73	0.425	3	0.266				
	1	74	0.991	7	0.481	21	4.389		
	2	74	0.850	6	0.566	14	0.651		
0103ZZ	3	73	5.182	4	3.398				
	4	73	6.286	2	6.088				
	5	73	3.370	2	2.888				
	6	73	3.568	5	3.341				
	7	73	1.926	9	2.605				
	8	73	1.586	6	1.671				
	9	73	1.416	4	1.416				
	10	73	1.019	12	0.906				
	11	73	1.529	6	1.274				
	12	73	1.812	3	1.189				
	1	74	3.681	7	1.954	21	13.734		
	2	74	3.256	6	2.265	14	2.549		

APPENDIX C

PHYSICAL AND CHEMICAL DATA

STORET RETRIEVAL DATE 76/07/22

010301
31 27 00.0 086 26 20.0 3
GANTT RESERVOIR
01039 ALABAMA

033392

11EPALES 2111202
0008 FEET DEPTH CLASS 00

DATE FROM TO	TIME OF DAY	DEPTH FEET	00010 WATER TEMP CENT	00300 DO MG/L	00077 TRANSP SECCHI INCHES	00094 CNDUCTVY FIELD MICROMHO	00400 PH SU	00410 T ALK CACO3 MG/L	00610 NH3-N TOTAL MG/L	00625 TOT KJEL N MG/L	00630 NO2&NO3 N-TOTAL MG/L	00671 PHOS-DIS ORTHO MG/L P
73/06/19	15	0000	26.9		18	45	6.80	19	0.180	0.600	0.200	0.012
	15	0004	25.6	5.4		47	6.80	20	0.140	0.400	0.200	0.005
73/08/30	12	0000	29.2	6.8	30	70	8.00	24	0.120	0.700	0.180	0.007
	12	0006	28.2	6.6		70	7.80	25	0.100	0.300	0.160	0.013
73/11/03	12	0000	18.1		36	87	7.50	32	0.070	1.000	0.170	0.011
	12	0005	16.8	8.6		84	7.60	34	0.050	0.300	0.250	0.014
	12	0011	15.9	8.4		87	7.70	37	0.060	0.200	0.280	0.010

DATE FROM TO	TIME OF DAY	DEPTH FEET	00665 PHOS-TOT MG/L P	32217 CHLRPHYL A UG/L
73/06/19	15	0000	0.052	2.7
	15	0004	0.049	
73/08/30	12	0000	0.022	3.0
	12	0006	0.027	
73/11/03	12	0000	0.030	0.9
	12	0005	0.032	
	12	0011	0.037	

STORET RETRIEVAL DATE 76/07/22

010302
31 26 15.0 086 28 45.0 3
GANTT RESERVOIR
01039 ALABAMA

033392

11EPALES 2111202
0018 FEET DEPTH CLASS 00

DATE FROM TO	TIME OF DAY	DEPTH FEET	00010 WATER TEMP CENT	00300 DO MG/L	00077 TRANSP SECCHI INCHES	00094 CNDUCTVY FIELD MICROMHO	00400 PH SU	00410 T ALK CACO3 MG/L	00610 NH3-N TOTAL MG/L	00625 TOT KJEL N MG/L	00630 NO2&NO3 N-TOTAL MG/L	00671 PHOS-DIS ORTHO MG/L P
73/06/19	15 55	0000	28.5		18	46	6.90	19	0.190	0.500	0.190	0.006
	15 55	0006	27.0	5.4		46	6.90	19	0.140	0.300	0.180	0.006
	15 55	0014	25.8	5.0		40	6.80	17	0.170	0.400	0.200	0.006
73/08/30	13 10	0000	31.1		38	61	7.80	20	0.120	0.500	0.160	0.009
	13 10	0005	29.8	5.0		60	7.60	21	0.130	0.400	0.110	0.010
	13 10	0015	28.3	2.6		68	7.40	18	0.200	0.400	0.130	0.008
73/11/03	12 20	0000	19.6		48	68	7.70	25	0.050	0.500	0.230	0.009
	12 20	0005	18.4	8.0		66	7.80	26	0.050	0.300	0.130	0.008
	12 20	0010	17.9	8.6		69	7.50	28	0.040	0.200	0.140	0.009

DATE FROM TO	TIME OF DAY	DEPTH FEET	00665 PHOS-TOT MG/L P	32217 CHLRPHYL A UG/L
73/06/19	15 55	0000	0.045	2.4
	15 55	0006	0.045	
	15 55	0014	0.054	
73/08/30	13 10	0000	0.026	1.7
	13 10	0005	0.023	
	13 10	0015	0.029	
73/11/03	12 20	0000	0.021	1.1
	12 20	0005	0.019	
	12 20	0010	0.029	

STORET RETRIEVAL DATE 76/07/22

010303
31 21 50.0 086 30 50.0 3
GANTT RESERVOIR
01039 ALABAMA
033391

11EPALES 2111202
0033 FEET DEPTH CLASS 00

DATE FROM TO	TIME OF DAY	DEPTH FEET	00010 WATER TEMP CENT	00300 DO MG/L	00077 TRANSP SECCHI INCHES	00094 CNDUCTVY FIELD MICROMHO	00400 PH SU	00410 T ALK CACO3 MG/L	00610 NH3-N TOTAL MG/L	00625 TOT KJEL N MG/L	00630 NO2&NO3 N-TOTAL MG/L	00671 PHOS-DIS ORTHO MG/L P
73/06/19	16 15	0000	28.8		24	46	6.90	33	0.140	0.600	0.180	0.010
	16 15	0006	27.7	6.4		46	6.90	23	0.130	0.400	0.170	0.005
	16 15	0015	26.7	4.4		47	6.80	20	0.150	0.400	0.200	0.005
	16 15	0022	25.8	4.0		47	6.80	20	0.210	0.400	0.200	0.006
	16 15	0029	25.2	3.2		48	6.70	20	0.210	0.400	0.200	0.006
73/08/30	13 25	0000	31.0		36	63	7.40	18	0.180	0.600	0.130	0.009
	13 25	0005	30.2	5.4		63	7.30	17	0.160	0.400	0.130	0.008
	13 25	0015	28.2	1.4		64	7.20	18	0.200	0.400	0.110	0.008
	13 25	0025	27.5	1.0		73	7.10	23	0.300	0.600	0.090	0.006
73/11/03	12 40	0000	21.4		60	67	7.30	24	0.040	0.300	0.120	0.007
	12 40	0005	19.5	8.2		64	7.40	22	0.040	0.200	0.120	0.008
	12 40	0015	19.1	7.0		64	7.30	23	0.040	0.200	0.120	0.008
	12 40	0025	19.0	9.0		64	7.30	23	0.050	0.200	0.130	0.008

DATE FROM TO	TIME OF DAY	DEPTH FEET	00665 PHOS-TOT MG/L P	32217 CHLRPHYL A UG/L
73/06/19	16 15	0000	0.045	2.1
	16 15	0006	0.044	
	16 15	0015	0.046	
	16 15	0022	0.049	
	16 15	0029	0.049	
73/08/30	13 25	0000	0.027	4.8
	13 25	0005	0.023	
	13 25	0015	0.026	
	13 25	0025	0.025	
73/11/03	12 40	0000	0.017	1.4
	12 40	0005	0.016	
	12 40	0015	0.019	
	12 40	0025	0.019	

APPENDIX D

TRIBUTARY DATA

STORET RETRIEVAL DATE 76/07/22

0103A1 LS0103A1
31 24 00.0 086 29 00.0 4
CONECUH RIVER
01 MAP COVINGTON CO
O/GANTT LAKE (RESVR) 033392
US 29 BRDG SE EDGE OF VLG OF GANTT
11EPALES 2111204
0000 FEET DEPTH CLASS 00

DATE FROM TO	TIME OF DAY	DEPTH FEET	00630 NO2&NO3 N-TOTAL MG/L	00625 TOT KJEL N MG/L	00610 NH3-N TOTAL MG/L	00671 PHOS-DIS ORTHO MG/L P	00665 PHOS-TOT MG/L P
73/03/04	10 30		0.091	0.340	0.032	0.008	0.025
73/04/02	09 30		0.054	0.980	0.240	0.013	0.045
73/05/02	13 50		0.069	1.380	0.068	0.012	0.045
73/06/05	13 45		0.154	2.200	0.168	0.018	0.045
73/07/09	15 20		0.096	1.400	0.130	0.013	0.035
73/08/06	10 45		0.120	0.380	0.096	0.010	0.040
73/09/04	09 00		0.050	0.540	0.136	0.005K	0.030
73/10/12	09 15		0.067	0.400	0.054	0.005K	0.020
73/11/06	14 15		0.100	0.650	0.038	0.005K	0.025
73/12/03	10 00		0.152	0.400	0.036	0.005	0.020
74/01/07	09 30		0.116	0.700	0.040	0.012	0.045
74/01/21	15 30		0.192	0.200	0.056	0.008	0.035
74/02/06	10 00		0.080	0.400	0.045	0.015	0.040
74/02/14	10 30		0.108	0.400	0.030	0.015	0.055

K VALUE KNOWN TO BE
LESS THAN INDICATED

STORET RETRIEVAL DATE 76/07/22

0103A2 LS0103A2
31 29 30.0 086 21 30.0 4
CONECUH RIVER
01 MAP COVINGTON CO
I/GANTT LAKE (RESVR) 033392
CO HWY 77 BRDG .75 MI SE OFDOZIER
11EPALES 2111204
0000 FEET DEPTH CLASS 00

DATE FROM TO	TIME OF DAY	DEPTH FEET	00630 NO2&NO3 N-TOTAL MG/L	00625 TOT KJEL N MG/L	00610 NH3-N TOTAL MG/L	00671 PHOS-DIS ORTHO MG/L P	00665 PHOS-TOT MG/L P
73/03/04	11 10		0.132	1.000	0.042	0.008	0.030
73/04/02	10 15		0.075	1.150	0.060	0.028	0.075
73/05/02	14 20		0.066		0.063	0.011	0.045
73/06/05	14 25		0.168	2.100	0.110	0.014	0.060
73/07/09	15 45		0.336	0.690	0.069	0.012	0.020
73/08/06	11 15		0.270	0.800	0.080	0.009	0.035
73/09/04	09 30		0.280	0.360	0.048	0.005K	0.020
73/10/12	09 45		0.250	0.270	0.037	0.008	0.030
73/11/06	15 00		0.264	0.550	0.054	0.008	0.020
73/12/03	10 40		0.116	0.400	0.024	0.012	0.012
74/01/07	10 00		0.088	1.400	0.036	0.012	0.050
74/01/21	15 45		0.140	0.500	0.048	0.012	0.060
74/02/06	10 50		0.080	0.400	0.040	0.015	0.040
74/02/14	11 20		0.048	0.500	0.030	0.015	0.060

K VALUE KNOWN TO BE
LESS THAN INDICATED

STORET RETRIEVAL DATE 76/07/22

010381 LS010381
31 28 00.0 086 24 30.0 4
BIG CREEK
01 MAP COVINGTON CO
T/GANTT LAKE (RESVR) 033392
US 29 BRDG 6.5 MI NE OF GANTT
11EPALES 2111204
0000 FEET DEPTH CLASS 00

DATE FROM TO	TIME OF DAY	DEPTH FEET	00630 NO2&NO3 N-TOTAL MG/L	00625 TOT KJEL N MG/L	00610 NH3-N TOTAL MG/L	00671 PHOS-DIS ORTHO MG/L P	00665 PHOS-TOT MG/L P
73/03/04	10 50		0.160	1.500	0.110	0.005K	0.017
73/04/02	10 30		0.105	0.800	0.046	0.005K	0.010
73/05/02	14 40						0.020
73/06/05	14 40		0.130	1.470	0.110	0.005K	0.025
73/07/09	16 00		0.176	2.000	0.105	0.006	0.015
73/08/06	11 00		0.160	0.260	0.054	0.005K	0.020
73/09/04	09 10		0.115	0.350	0.048	0.009	0.025
73/10/12	09 30		0.132	0.320	0.054	0.005K	0.030
73/11/06	14 30		0.092	0.300	0.042	0.005K	
73/12/03	10 20		0.152	0.300	0.052	0.005K	0.015
74/01/07	09 50		0.156	0.500	0.032	0.005K	0.010
74/01/21	15 45		0.208	0.350	0.060	0.005K	0.035
74/02/06	10 20		0.216	0.100K	0.035	0.005	0.005
74/02/14	10 50		0.208	0.200	0.040	0.005K	0.010

K VALUE KNOWN TO BE
LESS THAN INDICATED

STORET RETRIEVAL DATE 76/07/22

0103C1 Ls0103C1
31 25 00.0 086 26 30.0 4
FEAGIN CREEK
01 MAP COVINGTON CO
T/GANTT LAKE (RESVR) 033392
XING OF RD HEADIN NE OFF US29
11EPALES 2111204
0000 FEET DEPTH CLASS 00

DATE FROM TO	TIME OF DAY	DEPTH FEET	00630 NO2&NO3 N-TOTAL MG/L	00625 TOT KJEL N MG/L	00610 NH3-N TOTAL MG/L	00671 PHOS-DIS ORTHO MG/L P	00665 PHOS-TOT MG/L P
73/03/04	11 45		0.168	0.660	0.043	0.005K	0.020
73/04/02	09 55		0.189	1.000	0.084	0.007	0.025
73/05/02	14 00		0.180	0.780	0.072	0.007	0.025
73/06/05	14 00		0.138	1.980	0.118	0.013	0.055
73/07/09	15 30		0.168	0.330	0.042	0.005K	0.022
73/08/06	11 30		0.120	0.320	0.036	0.012	0.020
73/09/04	09 50		0.092	0.300	0.044	0.005K	0.020
73/10/12	10 00		0.115	0.480	0.052	0.005K	0.025
73/11/06	15 45		0.067	0.250	0.024	0.005K	0.020
73/12/03	11 00		0.112	0.300	0.040	0.005K	0.015
74/01/07	10 30		0.200	0.300	0.044	0.005K	0.020
74/01/21	16 15		0.300	0.300	0.052	0.016	0.065
74/02/06	11 15		0.224	0.200	0.045	0.005	0.015
74/02/14	11 45		0.216	0.300	0.070	0.005	0.020

K VALUE KNOWN TO BE
LESS THAN INDICATED

APPENDIX E

PARAMETRIC RANKINGS OF LAKES SAMPLED BY NES IN 1974 STATE OF ALABAMA

LAKE DATA TO BE USED IN RANKINGS

LAKE CODE	LAKE NAME	MEDIAN TOTAL P	MEDIAN INORG N	500-MEAN SEC	MEAN CHLORA	15-MIN DO	MEDIAN DISS ORTHO P
0101	BANKHEAD LAKE	0.029	0.770	452.667	4.017	14.900	0.007
0103	GANTT RESERVOIR	0.029	0.300	465.778	2.144	14.000	0.008
0104	GUNTERSVILLE RESERVOIR	0.044	0.480	461.111	8.567	12.200	0.014
0105	HOLT LOCK AND DAM	0.018	0.835	449.417	2.183	13.600	0.006
0106	LAY LAKE	0.076	0.390	470.778	7.056	13.000	0.032
0107	MARTIN LAKE	0.017	0.170	435.250	6.407	15.000	0.004
0108	MITCHELL LAKE	0.053	0.290	466.000	6.211	12.400	0.022
0109	PICKWICK LAKE	0.056	0.535	455.000	2.450	11.900	0.035
0112	WEISS RESERVOIR	0.092	0.260	478.389	11.261	14.900	0.034
0114	WILSON LAKE	0.053	0.460	447.714	7.400	10.200	0.022
0115	LAKE PURDY	0.049	0.170	437.889	12.711	15.000	0.014

PERCENT OF LAKES WITH HIGHER VALUES (NUMBER OF LAKES WITH HIGHER VALUES)

LAKE CODE	LAKE NAME	MEDIAN TOTAL P	MEDIAN INORG N	500-MEAN SEC	MEAN CHLORA	15-MIN DO	MEDIAN DISS ORTHO P	INDEX NO
0101	BANKHEAD LAKE	75 (7)	10 (1)	60 (6)	70 (7)	25 (2)	80 (8)	320
0103	GANTT RESERVOIR	75 (7)	60 (6)	30 (3)	100 (10)	40 (4)	70 (7)	375
0104	GUNTERSVILLE RESERVOIR	60 (6)	30 (3)	40 (4)	20 (2)	80 (8)	55 (5)	265
0105	HOLT LOCK AND DAM	90 (9)	0 (0)	70 (7)	90 (9)	50 (5)	90 (9)	390
0106	LAY LAKE	10 (1)	50 (5)	10 (1)	40 (4)	60 (6)	20 (2)	140
0107	MARTIN LAKE	100 (10)	95 (9)	100 (10)	50 (5)	5 (0)	100 (10)	450
0108	MITCHELL LAKE	40 (4)	70 (7)	20 (2)	60 (6)	70 (7)	35 (3)	295
0109	PICKWICK LAKE	20 (2)	20 (2)	50 (5)	80 (8)	90 (9)	0 (0)	260
0112	WEISS RESERVOIR	0 (0)	80 (8)	0 (0)	10 (1)	25 (2)	10 (1)	125
0114	WILSON LAKE	30 (3)	40 (4)	80 (8)	30 (3)	100 (10)	35 (3)	315
0115	LAKE PURDY	50 (5)	95 (9)	90 (9)	0 (0)	5 (0)	55 (5)	295

LAKES RANKED BY INDEX NOS.

RANK	LAKE CODE	LAKE NAME	INDEX NO
1	0107	MARTIN LAKE	450
2	0105	HOLT LOCK AND DAM	390
3	0103	GANTT RESERVOIR	375
4	0101	BANKHEAD LAKE	320
5	0114	WILSON LAKE	315
6	0115	LAKE PURDY	295
7	0108	MITCHELL LAKE	295
8	0104	GUNTERSVILLE RESERVOIR	285
9	0109	PICKWICK LAKE	260
10	0106	LAY LAKE	190
11	0112	WEISS RESERVOIR	125

U.S. DEPARTMENT OF COMMERCE
National Technical Information Service

PB-258 287

Biotransformation and Chemical Form of Mercury in Plants

Environmental Monitoring and Support Lab, Las Vegas, Nev

Jul 76

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO.	2.	3. RECIPIENT'S ACCESSION NO.
EPA-600/3-76-082		

4. TITLE AND SUBTITLE	5. REPORT DATE
BIOTRANSFORMATION AND CHEMICAL FORM OF MERCURY IN PLANTS	July 1976
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S)	8. PERFORMING ORGANIZATION REPORT NO.
Don D. Gay	
9. PERFORMING ORGANIZATION NAME AND ADDRESS Environmental Monitoring & Support Laboratory Office of Research and Development U.S. Environmental Protection Agency Las Vegas, NV 89114	10. PROGRAM ELEMENT NO. 1AA602
	11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS Same as above	13. TYPE OF REPORT AND PERIOD COVERED Final
	14. SPONSORING AGENCY CODE EPA-ORD, Office of Health and Ecological Effects

15. SUPPLEMENTARY NOTES

16. ABSTRACT

The in vitro methylation of inorganic mercury has been demonstrated using an acetone precipitated protein fraction from Pisum sativum and S-adenosyl-methionine as the methyl-donating compound.

A time course of the enzymatic formation of methylmercury has shown that the maximum methylmercury concentration occurred after 1 hour of incubation of the substrates with the enzyme system. At all subsequent incubation times reduced concentrations of methylmercury in the reaction mixture were observed.

When a time course for the in vivo methylation of inorganic mercury was done using an infiltration and incubation procedure, the maximum concentration of methylmercury was observed after 10 hours of incubation. All subsequent incubation periods produced lesser amounts of methylmercury.

The results suggest that the methylmercury is an intermediate compound in the mercury pathway in peas (Pisum sativum).

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Plant physiology	Methylmercury	06A
Biochemistry	Mercurial transformation	06C
Plant chemistry	Mercury uptake	06F
Mercury	Biotransformation	07B
Trace elements	Phenylmercury	07C
Leguminous plants	Pisum sativum	
Absorption		

PRICES SUBJECT TO CHANGE

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS (This Report)	21. NO. OF PAGES
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July 1976

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BIOTRANSFORMATION AND CHEMICAL FORM OF MERCURY IN PLANTS

by

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INTRODUCTION

The continuing release of mercury to the biosphere from natural as well as manmade sources warrants the study of plant exposures to mercury. Data on the effects of mercury pollution, the uptake and distribution of mercury, the transformation and identification of mercury species and the possibility of cycling of mercury in and through plants are vitally needed to complete the environmental assessment of this pollutant.

Many investigators, Aarkrog and Lippert (1971), Alvarez (1974), Barker (1972), Gerdes et al. (1974), John (1972), Lee et al. (1972), Smart (1964), Smith (1972), Tkachuk and Kuzina (1972), Van Loon (1974a and 1974b), and Yamada (1968), have reported the uptake and distribution of mercury in a variety of plant species.

Recently, the loss of metals from plants has been investigated. Siegel et al. (1974) reported on the loss of a volatile form of mercury from plants. The plants were harvested and the mercury content of fractions from the total number of plants harvested was determined. It was found that fractions of plants analyzed immediately after harvesting contained more mercury than plant fractions analyzed at later times. The suspected volatile form of mercury was found to be very soluble in hexane, less soluble in methanol and least soluble in water. This volatile mercury compound is not methylmercury or dimethylmercury.

Beauford et al. (1975) have obtained evidence that higher plants may contribute heavy metals naturally to the atmosphere. Peas, broad beans, and pine seedlings were grown in solutions of radioactive zinc, lead, or copper. A small fraction of the radioisotopes absorbed by the roots was released from the leaves. The metal release from the leaves was not found to be quantitatively associated with water loss. However, there was a requirement that the plants be continuously fed with the radioisotope. Removal of the plants from the radioactive solutions resulted in a complete cessation of radioisotope loss. All three metals were released from the plants but not in equivalent amounts per unit of tissue.

The methylation of mercury by aquatic organisms has been shown by Jensen and Jernelov (1969), Langley (1973), and Bisogni and Lawrence (1973). Wood et al. (1968) showed the methylation of mercury by methanogenic bacteria. Imura et al. (1972) showed the methylation of mercury by a liver homogenate from tuna. The role of methylmercury in these investigations has not been clearly elucidated.

Various investigators, Imura et al. (1971), Ingraham (1964), Wood et al. (1968), Wood (1974), and Bertilsson and Neujahr (1971) have used methylcobalamin as a methyl-donating compound. Wood et al. (1968) found

that the transfer of the methyl group from methylcobalamin to mercury could be accomplished non-enzymatically in mild reducing conditions.

Methylcobalamin is not the only biological compound capable of donating a methyl group in biological reactions. Cantoni (1955, 1957, and 1975) and Mudd (1973) have examined S-adenosyl-methionine as a methyl-donating compound and have found that it effectively methylates amino acids, proteins, and carbohydrates in biological reactions.

In a previous study (Gay, 1975) I reported the formation of methylmercury in Pisum sativum under several conditions; i.e., when peas were sprayed with a solution of mercuric nitrate, when peas were grown in soil containing mercuric nitrate or phenylmercuric acetate and when pea tissues were infiltrated and incubated in solutions of mercuric nitrate or phenylmercuric acetate. It was noted that very low concentrations of methylmercury were found and that younger peas produced more methylmercury than did older peas.

The purpose of this study was to investigate the mechanism of the methylation of mercury compounds in peas and to determine whether methylmercury is the end product in this pathway.

CONCLUSIONS

In vitro methylation of inorganic mercury has been accomplished by an enzyme system isolated from Pisum sativum. The methyl-donating compound was found to be S-adenosyl-methionine.

Formation of methylmercury in the peas is viewed as an intermediate step in the biochemical pathway of mercury in peas.

MATERIALS AND METHODS

The common dwarf garden pea, Pisum sativum cultivar Little Marvel, was used in all investigations. The seeds were planted in vermiculite and harvested 13 to 14 days after planting.

The infiltration and incubation procedure and analytical methodologies are discussed in detail elsewhere (Gay, 1975). The selected tissues are placed in a mercury solution and infiltrated under partial vacuum for 15 minutes. The tissues are then incubated at normal pressure for various periods of time in complete darkness.

A Hewlett-Packard gas chromatograph equipped with a nickel-63 linearizing electron capture detector is used to detect the presence and quantities of methylmercury in the samples.

Partial purification of enzyme systems was accomplished with an acetone protein precipitation procedure. The amount of acetone added to obtain the various fractions is expressed as the percent of acetone in the supernatant solution. Spectral grade acetone was used in all cases.

After harvesting the selected tissues, all procedures up to the incubations of enzyme fractions with the substrates were done at -4° to 2° C.

The supernatant solution obtained from the centrifugation of the ground tissues was treated successively with 25%, 50%, 70%, and 92% acetone. The acetone for each fraction was added dropwise to the supernatant solution while the solution was being agitated or stirred. The stirring continued 15 minutes after the addition of acetone. The mixture was then centrifuged at 15,000 rpm for 30 minutes at -4° C. The supernatant solution was treated with the next amount of acetone, consecutively, and the pellet obtained was placed under a stream of nitrogen to drive off the acetone. The pellet was dissolved in a small volume of buffer and used as an enzyme preparation.

All reaction mixtures, consisting of the substrates and the enzyme system, were incubated at 30° C, in a constant temperature bath for the duration of the incubation period.

RESULTS AND DISCUSSION

A time course of methylmercury formation in pea stems via the infiltration and incubation procedure was carried out to determine the incubation time required for the formation of the maximum concentration of methylmercury. Ten grams of stems from the peas grown for 14 days in the greenhouse were used for each time determination. The infiltration and incubation solution contained 10 parts per million mercuric nitrate. The results obtained for the time course (Figure 1) show that the maximum formation of methylmercury occurred after 10 hours of incubation and declined to approximately 25% of the maximum after 20 hours of incubation. These findings suggest that methylmercury is either lost by the tissues or it is transformed to another form of mercury which cannot be detected with the equipment and methodologies used.

In our previous study (Gay, in press) an experiment was set up to determine whether methylmercury or ethylmercury was being liberated from the incubation mixture. A nitrogen stream was directed into the incubation solution and subsequently into a carbonate-phosphate trap as described by Kimura and Miller (1960) and into a cysteine trap. After 20 hours of incubation, the traps were analyzed. No methylmercury or ethylmercury was detected. The total mercury balance in the infiltrated and incubated tissues has not been determined at this time, so the question of the transformation of methylmercury to a volatile mercury species which is lost or the transformation of methylmercury to a species which is not detected cannot be answered at this time.

The supernatant solution obtained after the centrifugation of pea tissues ground in 0.01M phosphate buffer with a mortar and pestle was used initially as a crude enzyme preparation. Methionine was selected as the first compound to be used as a substrate for a methyl-donating compound with which to methylate mercuric nitrate. After various periods of incubation, no methylmercury was found. Several cofactors were added in various combinations and molarities in an attempt to complete the enzyme system.

Methylmercury was formed when 6 milliliters of a supernatant solution, obtained by grinding 1 gram of tissue per 5 milliliters of phosphate buffer, was incubated for 4 hours at 30° C, in the dark with magnesium, manganese, ATP, methionine and mercury in the following final concentrations: 4×10^{-4} M magnesium and manganese, 1×10^{-2} M ATP, 2.5×10^{-4} M methionine, and 5.5×10^{-5} M mercuric nitrate.

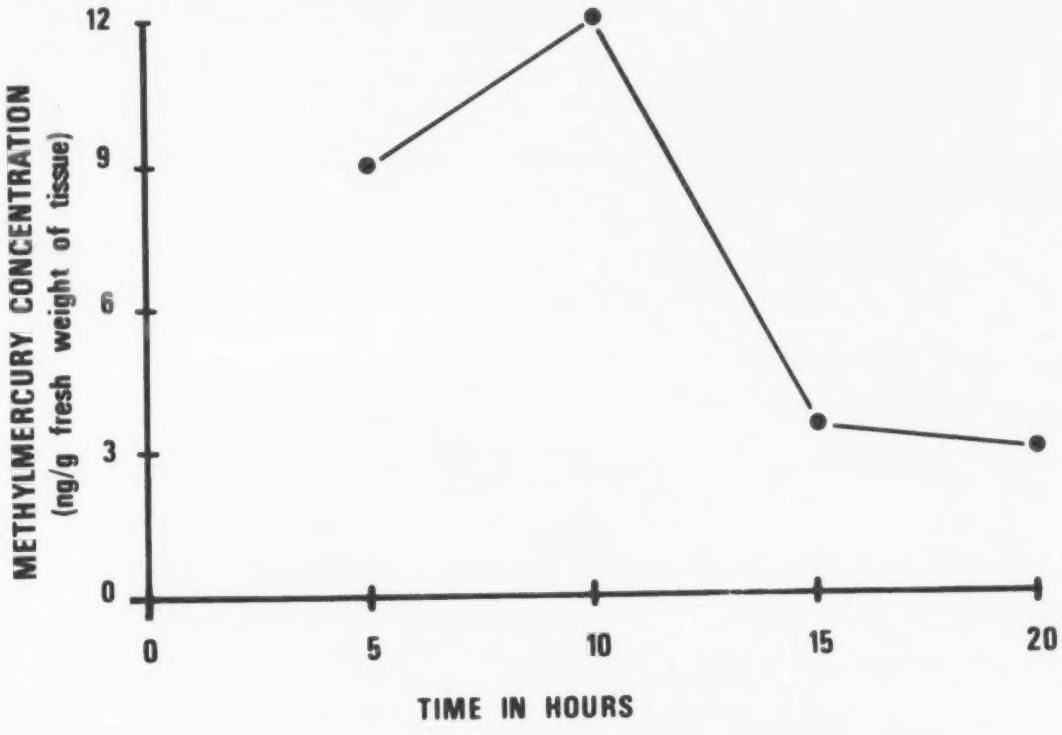

Figure 1. Time course for the formation of methylmercury in the infiltration and incubation procedure.

This same incubation mixture was used to determine the optimum pH of the enzyme system which forms methylmercury. An equal weight of tissues from peas grown for 14 days in the greenhouse was used for each of five pH values being tested. The tissues were ground in a specific pH buffer and the various cofactors were dissolved in the same buffer. After a 4-hour incubation of each at 30° C, the incubation mixture was analyzed for methylmercury. Maximum formation of methylmercury under this set of conditions occurred at pH 7.5 (Figure 2).

Two possibilities exist for the mechanism involved in the enzymatic, in vitro, methylation of mercuric nitrate with methionine and the various cofactors used. The methylation reaction may be straightforward, utilizing methionine as the methyl-donating compound but requiring the cofactors to complete the enzyme system methylating mercury. The other possibility is

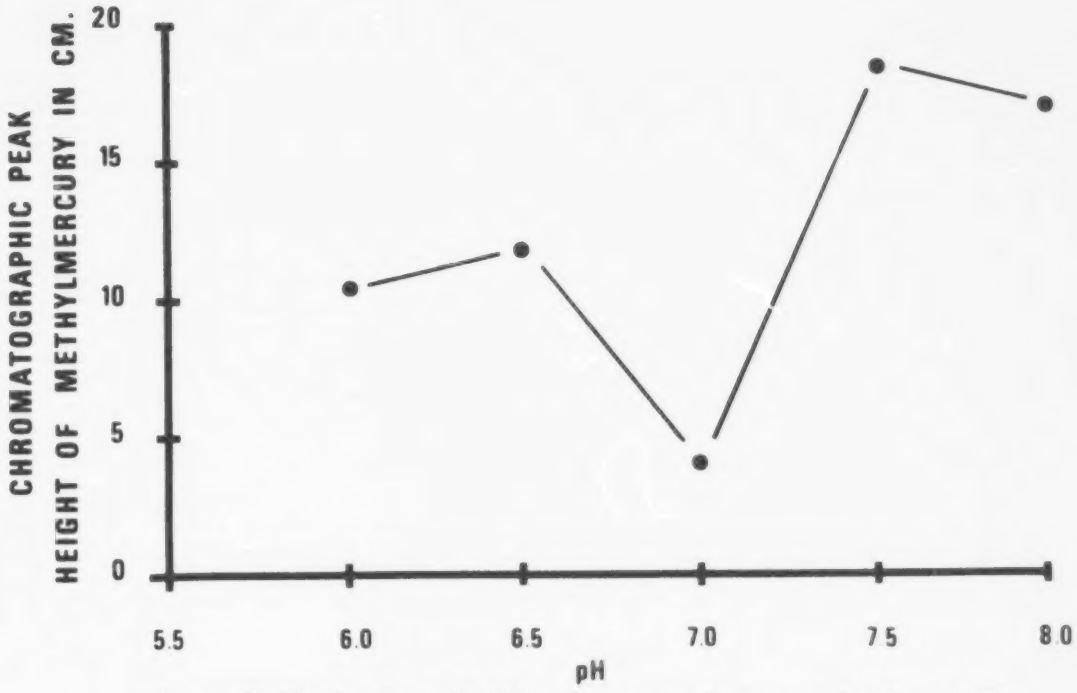

Figure 2. The in vitro methylation of mercuric nitrate as a function of pH.

that the cofactors plus methionine are involved in an enzymatic reaction forming S-adenosyl-methionine which, when formed, reacts with the mercuric nitrate enzymatically to form methylmercury. The latter possibility is the more plausible because the specific cofactors, the concentrations of the cofactors used, and the optimum pH for methylmercury formation are extremely similar to those which Cantoni (1955 and 1957) found to be required for the enzymatic synthesis of S-adenosyl-methionine.

The acetone protein precipitation procedure was used to partially purify the enzyme system forming methylmercury. Each protein fraction from the acetone precipitation procedure was incubated with methionine, mercuric nitrate and the various cofactors for 4 hours at pH 7.5. After the incubation period, each fraction was analyzed for methylmercury. The proteins precipitated between 70% and 92% acetone addition contained the enzyme system which forms methylmercury.

Because of the possibility of S-adenosyl-methionine being the methyl-donating compound, S-adenosyl-methionine (Sigma Chemical Company) was used as a substrate along with mercuric nitrate. Proteins precipitated between 70% and 92% acetone were divided into two equal volumes. One was incubated with methionine and mercuric nitrate, and the other was incubated with S-adenosyl-methionine and mercuric nitrate. The final concentrations of methionine and S-adenosyl-methionine were 1×10^{-3}M, and the final concentration of mercuric nitrate in each was 1.1×10^{-4}M. The incubation period was 4 hours at 30° C. Each incubation mixture was analyzed for methylmercury. S-adenosyl-methionine as substrate produced a relative concentration of 25 for methylmercury, while methionine as substrate produced a relative concentration of 1, barely distinguishable from background.

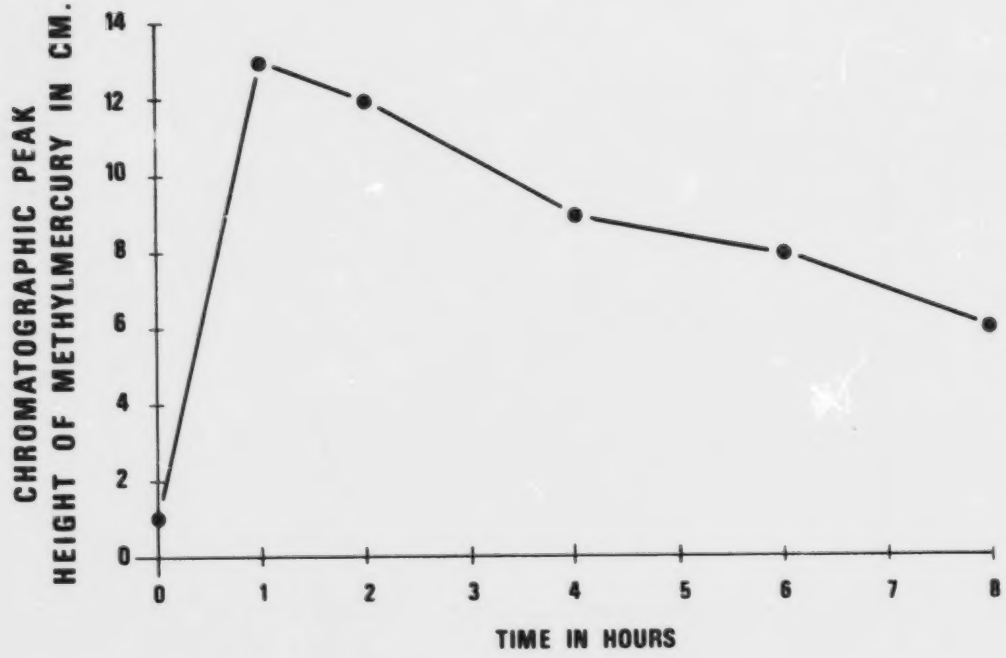

Figure 3. Time course for the in vitro methylation of mercuric nitrate.

A time course over an 8-hour period was done to determine when the maximum amount of methylmercury was formed with S-adenosyl-methionine as the methyl-donating substrate. Sixty grams of stems and apices from peas 14 days old were harvested. The tissues were ground in 0.01M phosphate buffer at pH 7.5 using 1.5 milliliter buffer per gram fresh weight of tissue. The 70% to 92% acetone-precipitated proteins were dissolved in 20 milliliters of phosphate buffer. S-adenosyl-methionine at a final concentration of 5×10^{-4}M and mercuric nitrate at a final concentration of 1.1×10^{-4}M were added to the protein solution. Aliquots were removed at 0, 1, 2, 4, 6, and 8 hours. Each aliquot was immediately analyzed for methylmercury. Maximum formation of methylmercury occurred after 1 hour of incubation with a steady decline in amount of methylmercury thereafter to 8 hours (Figure 3).

The results correspond to the time course obtained from the infiltration and incubation experiment as shown in Figure 1. In both in vivo and in vitro experiments methylmercury concentrations reached a peak and then declined thereafter. What happens to the methylmercury is unknown at this time, but this study suggests that methylmercury is an intermediate product in the mercury pathway in peas.

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